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## **Impact of HIV-1 reverse transcriptase polymorphism F214L on virological response to thymidine analogue-based regimens in antiretroviral therapy (ART)–naïve and ART-experienced patients**

Ceccherini-Silberstein, Francesca ; Cozzi-Lepri, Alessandro ; Ruiz, Lidia ; Mocroft, Amanda ; Phillips, Andrew N ; Olsen, Christian Holkmann ; Gatell, Jose M ; Günthard, Huldrych F ; Reiss, Peter ; Perno, Carlo Federico ; Clotet, Bonaventura ; Lundgren, Jens D

**Abstract:** Background.160;A negative association between the polymorphism F214L and type 1 thymidine analogue (TA) mutations (TAMs) has been observed. However, the virological response to TAs according to the detection of F214L has not been evaluated. **Methods.**We studied 590 patients from EuroSIDA who started TA therapy for the first time as part of potent combination antiretroviral therapy (cART) and who were tested for genotypic resistance within the past 6 months. End points were median reduction in the week 24 viral load and time to virological failure (2 consecutive VL measurements >400 copies/mL after at least 6 months of the TA-containing cART). **Results.**In ART-naïve patients, the prevalence of F214L was 17%. By 48 months after starting TA-based cART, the proportion of patients who experienced virological failure was 16% in patients with 214L and 36% in those with 214F ( $P = .03$ ). In a multivariable Cox regression model, the relative hazard of virological failure for patients with 214L compared with those with 214F was 0.22 (95% confidence interval, 0.07–0.72). In ART-experienced patients, results were similar, and larger differences in virological response associated with the detection of 214L versus F were observed in patients with M41L/T215Y and mixed TAM profiles detected before the initiation of cART. **Conclusions.**This study provides evidence that the detection of polymorphism F214L is associated with a favorable virological response to TA-based cART

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# Impact of HIV-1 Reverse Transcriptase Polymorphism F214L on Virological Response to Thymidine Analogue–Based Regimens in Antiretroviral Therapy (ART)–Naïve and ART-Experienced Patients

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**Background.** A negative association between the polymorphism F214L and type 1 thymidine analogue (TA) mutations (TAMs) has been observed. However, the virological response to TAs according to the detection of F214L has not been evaluated.

**Methods.** We studied 590 patients from EuroSIDA who started TA therapy for the first time as part of potent combination antiretroviral therapy (cART) and who were tested for genotypic resistance within the past 6 months. End points were median reduction in the week 24 viral load and time to virological failure (2 consecutive VL measurements >400 copies/mL after at least 6 months of the TA-containing cART).

**Results.** In ART-naïve patients, the prevalence of F214L was 17%. By 48 months after starting TA-based cART, the proportion of patients who experienced virological failure was 16% in patients with 214L and 36% in those with 214F ( $P = .03$ ). In a multivariable Cox regression model, the relative hazard of virological failure for patients with 214L compared with those with 214F was 0.22 (95% confidence interval, 0.07–0.72). In ART-experienced patients, results were similar, and larger differences in virological response associated with the detection of 214L versus F were observed in patients with M41L/T215Y and mixed TAM profiles detected before the initiation of cART.

**Conclusions.** This study provides evidence that the detection of polymorphism F214L is associated with a favorable virological response to TA-based cART.

To date, a number of mechanisms are known to contribute to decreased HIV susceptibility to nucleoside reverse transcriptase inhibitors (NRTIs) [1–8]. One of

these mechanisms consists of promoting the selective excision of the incorporated nucleoside analogue from the terminated DNA chain and is determined by a group of mutations named “thymidine analogue (TA) mutations” (TAMs; M41L, D67N, K70R, L210W, T215Y/F, and K219E/Q). These mutations occur very

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frequently, predominantly under the pressure of antiretrovirals such as zidovudine and stavudine, and cause cross-resistance to other NRTIs [4–7].

Data from several studies suggest that TAMs are found in 2 distinct clusters defined by different mutation patterns (type 1 TAMs include M41L, L210W, and T215Y, and type 2 TAMs include D67N, K70R, T215F, and K219Q/E) [9–12], and recent statistical analyses have confirmed that the clustering is a real phenomenon [13]. Nonetheless, this distinction should not be considered absolute because several mutations (especially M41L and D67N) from one group can coexist with those from the other [13, 14].

Factors that drive the selection of type 1 TAMs versus type 2 TAMs have not been thoroughly investigated. These might include the genetic background of HIV-1 reverse transcriptase (RT), immune selection pressure, and the particular type, sequence, and duration of TA use [13].

The prevalence of type 1 TAMs seems to be higher than that of type 2 TAMs [9, 13, 14], the type 1 TAM profile is associated with higher phenotypic resistance to NRTIs than the type 2 TAM profile [15], and type 1 TAMs are associated with impaired virological response to tenofovir-containing therapy, whereas type 2 TAMs do not have such a detrimental effect [16, 17]. However, mutations such as M41L, M184V, L210W, and T215Y have been associated with a better, although transient, virological outcome in patients treated with efavirenz-based regimens [18].

F214L is a natural polymorphism detected in ~18% of antiretroviral therapy (ART)–naïve and treated individuals [19]. Recently, a strong negative association between F214L and type 1 TAMs and, vice versa, a positive association between F214L and type 2 TAMs have been observed [20]. Nevertheless, the correlation between this polymorphism and virological response to therapy in individuals starting combination ART (cART) has not yet been investigated. The objectives of the present analysis were as follows: first, to evaluate the prevalence of F214L in the EuroSIDA cohort and its association with RT resistance mutations and patterns of mutations; second, to assess the virological response to a TA-based cART according to the detection of F214L alone or concomitantly with specific TAMs in both ART-naïve and ART-experienced patients.

## METHODS

**Study population.** Patients included were those starting zidovudine or stavudine therapy for the first time while concomitantly receiving  $\geq 2$  other antiretrovirals (cART) and who had been tested for genotypic resistance in the 6 months before starting cART. Some patients were already TA experienced, but only as mono- or dual-therapy before cART.

The majority of the genotypic tests ( $n = 583$ ; 98.8%) used in this analysis were performed retrospectively on stored plasma

in a central laboratory in Spain using standardized equipment—the Trugene HIV-1 Genotyping Kit and the OpenGene DNA Sequencing System (version 8.0)—in accordance with the manufacturer’s recommendations. The remaining 1.2% of tests had been performed at laboratories linked to the clinical sites at which the patient was being followed up. All nucleotide sequences have been deposited in GenBank; accession numbers are EF563151–EF563824.

Phylogenetic analysis of the RT sequences was performed on a subset of the included patients to determine HIV subtype. We considered only RT mutations that have been associated with zidovudine and/or stavudine resistance and listed by a panel of European experts [21] plus mutations M184I/V, which are known to confer hypersusceptibility to zidovudine and stavudine [5, 22, 23]. Type 1 and type 2 TAM profiles were defined similarly to other previous analyses [13, 14]. In particular, 2 definitions were used. One was a simple definition, with 1 or more changes among M41L, L210W, and T215Y (with or without D67N and no K70R or K219E/Q) being classified as a type 1 TAM profile. Similarly, the type 2 TAM profile was defined as including 1 or more mutations among D67N, K70R, T215F, and K219E/Q (and no M41L, L210W, or T215Y). Patients with both type 1 and type 2 TAMs were classified in the mixed TAM profile group and were typically patients with a large number of TAMs. A second, stricter definition grouped patients according to the detection of any combination of  $\geq 2$  mutations among M41L, L210W, and T215Y (with or without D67N and no type 2 TAMs) as a type 1 TAM profile; similarly, the type 2 TAM profile was classified by any combination of  $\geq 2$  mutations among D67N, K70R, T215F, and K219E/Q (and no type 1 TAMs).

**Statistical analysis.** Associations between mutations were tested using the  $\chi^2$  test and Fisher’s exact test, as appropriate. No adjustment was used to control for multiple-hypothesis testing; however, because, in addition to polymorphism 214L, mutations at 17 other codons of the RT region were investigated, associations were considered to be statistically significant at  $P < .003$  (the conservative method of Bonferroni) [24].

The virological response to therapy was evaluated using 2 separate approaches: analysis of the median reduction in the week 24 viral load (VL) and time to virological failure. Week 24 VL reduction was calculated as the difference between the VL measured at the time of starting the TA-containing cART and the VL measured in a time window between 16 and 32 weeks. Because 26% of the patients had an undetectable VL at this time point, the median VL reduction, both overall and in subgroups, was calculated using the Kaplan-Meier approach to account for the censored observations [25]. Accordingly, the adjusted effect of specific mutations and of sets of mutations on the week 24 VL reduction was evaluated using a linear regression model that accounts for truncated values.

Time to virological failure was defined as the time from starting cART to the first of 2 consecutive VL measurements >400 copies/mL after at least 6 months from the initiation of the TA-containing cART. The follow-up of patients who did not experience virological failure was censored at the time of the last available VL. The analysis was conducted according to an intention-to-continue-treatment principle by ignoring changes in therapy. Kaplan-Meier estimates and proportional hazards Cox regression models were used to test the prognostic value of mutation F214L and other factors for predicting time to virological failure. The Cox regression model was stratified by clinical center. Survival analyses were repeated using an on-treatment approach by censoring patients' follow-up at the time when the TA was interrupted (referred to as "on-treatment analyses"). See the footnote of table 3 for a complete list of potential confounders used in the multivariable analyses. In the linear regression analysis, the exact number of weeks between the date of starting cART and the week 24 VL measurement was an additional covariate.

On the basis of previous findings [20, 26, 27], we tested in ART-experienced patients the hypotheses that the virological response associated with the detection of 214L (vs. 214F) could be different according to the concomitant detection of different mutations (specific TAMs or TAM profiles).

## RESULTS

**Characterization of the study population.** The present analysis includes 590 patients from EuroSIDA, who started zidovudine or stavudine therapy for the first time as part of cART; 247 patients (42%) started zidovudine-containing regimens, the remaining 343 (58%) started stavudine. The most frequently used drugs besides the TAs were lamivudine ( $n = 457$  [77%]), indinavir ( $n = 166$  [28%]), ritonavir ( $n = 161$  [27%]), didanosine ( $n = 91$  [15%]), and nevirapine ( $n = 81$  [14%]). Overall, the types of TA-containing cART were 3 NRTIs ( $n = 28$  [5%]), 2 NRTIs plus a single protease inhibitor (PI) ( $n = 291$  [49%]), 2 NRTIs plus a nonnucleoside reverse transcriptase inhibitor ( $n = 84$  [14%]), 2 NRTIs plus a ritonavir-boosted PI ( $n = 82$  [14%]), and other combinations containing 3 antiretrovirals ( $n = 40$  [7%]), 4 antiretrovirals ( $n = 46$  [8%]), and >4 antiretrovirals ( $n = 19$  [3%]).

The genotypic test was performed on average 1 month (range, 0–6 months) before the initiation of the TA-containing cART regimen (range, June 1995–June 2005).

Two hundred thirty-six patients (40%) were antiretroviral naive when they were tested. For the remaining 354 patients, the median number of antiretrovirals previously used was 3 (interquartile range [IQR], 2–4).

**Prevalence of resistance mutations before the initiation of TA-based cART.** Using the simple definitions, we grouped patients according to the observed pattern of TAMs as follows:

334 patients with no TAMs (57%), 137 (23%) with type 1 TAMs, 59 (10%) with type 2 TAMs, and 61 (10%) with a mixed profile. Overall, polymorphism F214L was detected in 99 patients (17%). Table 1 shows the prevalence of F214L and RT resistance mutations, both overall and according to patients' treatment history. As expected, the prevalence of resistance mutations was much higher in ART-experienced than ART-naïve patients.

There was evidence for a difference in the prevalence of F214L in patients with no previous exposure to TA (5/22 [23%]) or previous exposure to zidovudine alone (41/284 [14%]) and with previous exposure to both zidovudine and stavudine (14/48 [29%]) ( $P = .03$ ). The prevalences of TAMs in zidovudine-experienced patients were as follows: M41L (47%), D67N (37%), K70R (33%), L210W (35%), T215F (7%), T215Y (53%), K219E (6%), and K219Q (13%). In stavudine-experienced patients, TAM prevalences were as follows: M41L (39%), D67N (22%), K70R (20%), L210W (22%), T215F (7%), T215Y (41%), K219E (2%), and K219Q (13%).

In ART-naïve patients, the only significant association between the detection of F214L and all other RT mutations considered was for V118I (118I was present in 7 of 39 [18%] of those with 214L vs. 2 of 197 [1%] of those with 214F;  $P = .0003$ ).

In ART-experienced patients, several associations between variant L/F at position 214 and TAMs were observed (figure 1). This analysis confirms previous observations that F214L is less frequently detected in patients with type 1 TAMs or mixed profiles than in those with type 2 TAMs or no TAMs.

HIV subtype data were available for 218 (92%) of the total 236 ART-naïve patients, of whom 30 (14%) carried non-B subtypes (4% A, 5% C, 0.5% D, 0.5% G, and 4% circulating recombinant forms). No difference in the prevalence of 214L in patients with subtype B versus patients with subtype non-B was found.

**Virological response to cART.** In 236 patients who were ART naïve when they started the TA-containing cART, the overall average reduction in week 24 VLs after the initiation of therapy was 2.77 (95% confidence interval [CI], 2.50–2.95)  $\log_{10}$  copies/mL. This reduction was 0.48 (95% CI, –0.16 to 1.12;  $P = .14$ )  $\log_{10}$  copies/mL greater in patients with 214L than in those with 214F, after controlling for confounders in a linear regression model. By 48 months after starting the TA-based cART, the proportion of patients who experienced virological failure was 16% (95% CI, 4%–28%) in patients with the 214L variant and 36% (95% CI, 28%–44%) in those with the 214F variant ( $P = .03$ ) (figure 2). The median number of VL measurements during follow-up was similar in the 2 groups: 3.4 (IQR, 2.5–4.1) per year in patients with 214F and 3.2 (IQR, 2.3–4.1) per year in those with 214L.

In the Cox regression model, the relative hazard (RH) of

**Table 1. Prevalence of polymorphism F214L and a no. of drug-resistance mutations in the reverse transcriptase (RT) region, according to patients' treatment history.**

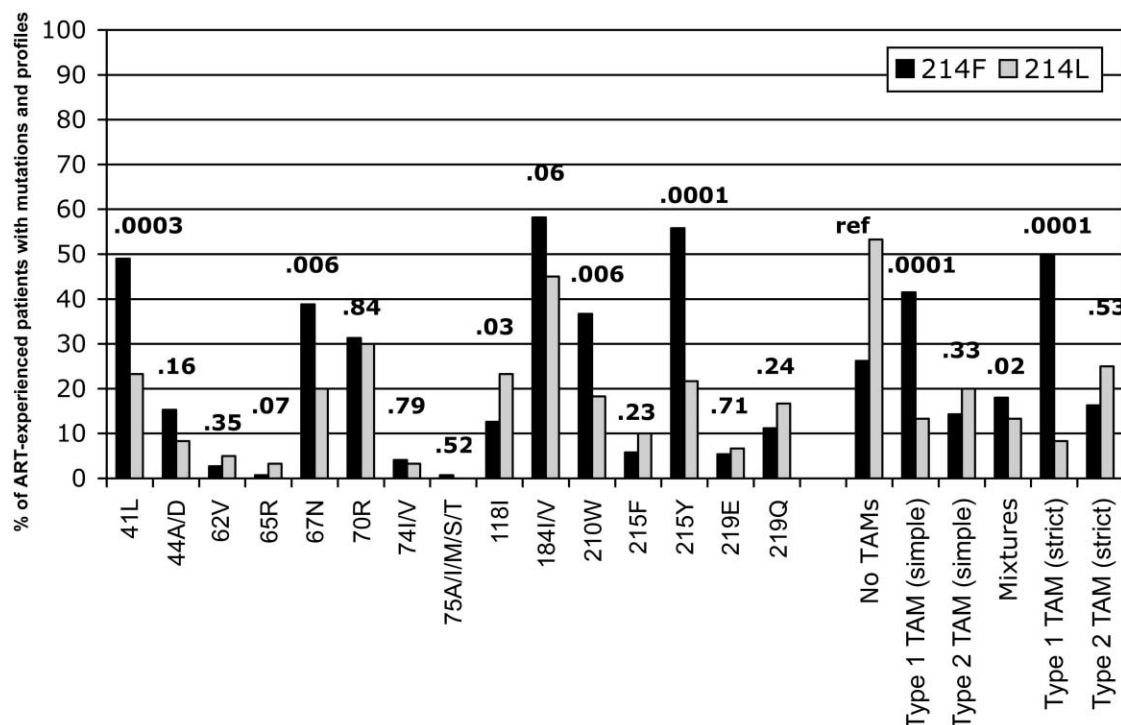
Mutation	ART status		Total	P
	Experienced	Naive		
RT mutation				
41L	158 (44.6)	7 (3.0)	165 (28.0)	.0001
44A/D	50 (14.1)	2 (0.9)	52 (8.8)	.0001
62V	11 (3.1)	2 (0.9)	13 (2.2)	.09
65R	4 (1.1)	0 (0.0)	4 (0.7)	.15
67G/N	127 (35.9)	6 (2.5)	133 (22.5)	.0001
70R	110 (31.1)	2 (0.9)	112 (18.9)	.0001
74I/V	14 (4.0)	1 (0.4)	15 (2.5)	.008
75A/I/M/S/T	2 (0.6)	0 (0.0)	2 (0.3)	.52
77L	3 (0.9)	0 (0.0)	3 (0.5)	.28
116Y	1 (0.3)	0 (0.0)	1 (0.2)	1.00
118I	51 (14.4)	9 (3.8)	60 (10.2)	.0001
151M	1 (0.3)	0 (0.0)	1 (0.2)	1.00
184I/V	198 (55.9)	5 (2.1)	203 (34.4)	.0001
210W	119 (33.6)	4 (1.7)	123 (20.9)	.0001
214L	60 (17.0)	39 (16.5)	99 (16.8)	.89
215F	23 (6.5)	1 (0.4)	24 (4.1)	.0003
215Y	177 (50.0)	3 (1.3)	180 (30.5)	.0001
219E/H/N/Q/R	73 (20.6)	6 (2.5)	79 (13.4)	.0001
TAM profile				
No TAMs	109 (30.8)	225 (95.3)	334 (56.6)	.0001
Simple definition				
Type 1 TAM	130 (36.7)	7 (3.0)	137 (23.2)	.0001
Type 2 TAM	54 (15.3)	4 (1.7)	58 (9.8)	.0001
Mixed TAM	61 (17.2)	0 (0.0)	61 (10.3)	.0001
Strict definition				
Type 1 TAM	117/275 (43.0)	7/236 (3.0)	124/511 (24.3)	.0001
Type 2 TAM	49/275 (17.8)	4/236 (1.7)	53/511 (10.4)	.0001
Total	354	236	590	

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. *P* values are from a  $\chi^2$  test (or Fisher's exact test, when appropriate) to test the hypothesis that the prevalence of specific mutations or profiles is different between antiretroviral therapy (ART)-naive and ART-experienced patients. TAM, thymidine analogue mutation.

virological failure for patients with 214L compared with those with 214F was 0.22 (95% CI, 0.07–0.72; *P* = .01) (table 2). Also, older patients tended to be less likely to experience virological failure (RH per 10 years older age, 0.77 [95% CI, 0.58–1.01]; *P* = .06). No significant difference in virological response was found between patients starting a zidovudine-containing cART and those starting a stavudine-containing cART (*P* = .90). Also, there was no evidence that the difference in virological response associated with the detection of 214L versus 214F was different according to which TA was started (RH for 214L vs. 214F, 0.10 in those starting zidovudine therapy vs. 0.18 in those starting stavudine therapy; *P* for interaction = .44). Results were similar when we repeated the analysis using the on-treatment approach (data not shown).

In ART-experienced patients, the virological response was also better in patients with variant 214L than in those with 214F. The week 24 VL reduction was 1.75 log<sub>10</sub> (95% CI, 0.98–3.00) copies/mL in patients with 214L and was 1.35 (95% CI, 1.08–1.64) log<sub>10</sub> copies/mL in those with 214F (*P* = .02, log-rank test). In the multivariable analysis, patients with 214L had a greater VL reduction from baseline than those with 214F, although it was not statistically significant (adjusted mean differences of 214L vs. 214F, 0.19 [95% CI, –0.28 to +0.65]; *P* = .44).

By 48 months after starting the TA-based cART, the proportion of patients who experienced virological failure was 47% (95% CI, 33%–61%) in patients with 214L and was 72% (95% CI, 66%–78%) in those with 214F (*P* = .007). The median



**Figure 1.** Proportion of patients with reverse transcriptase mutations and mutational profiles, according to concomitant detection of 214F or 214L polymorphism. *P* values shown above the bars are from  $\chi^2$  tests; associations are considered to be statistically significant if  $P < .003$ . TAM, thymidine analogue mutation.

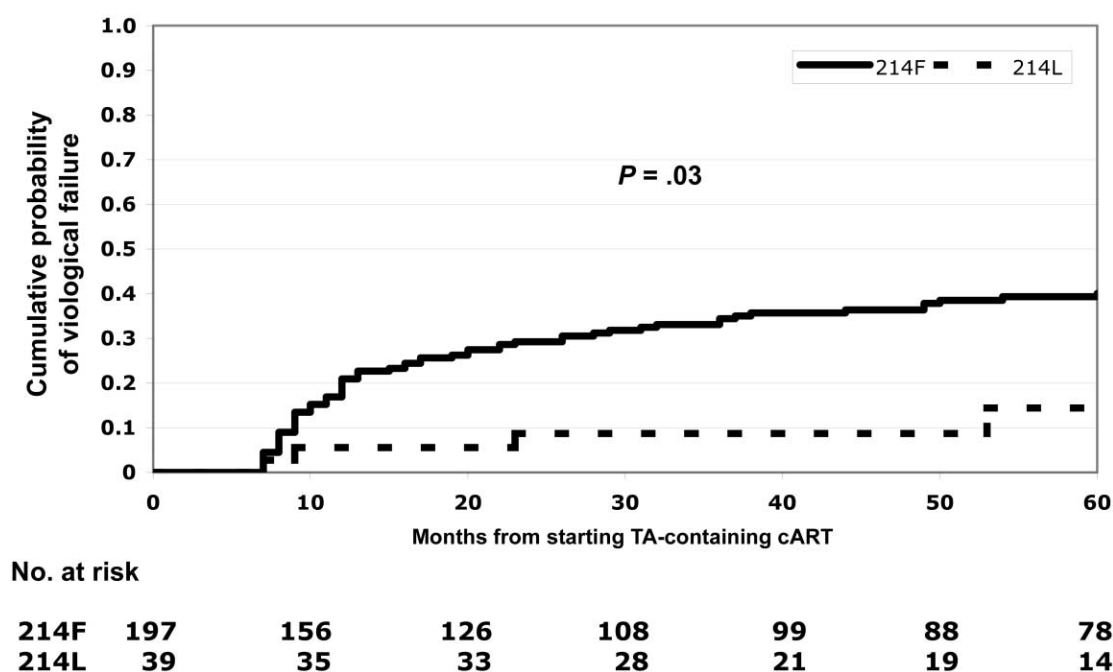
number of VL measurements during follow-up was 4.0 (IQR, 3.4–5.1) in patients with 214F and was 4.0 (IQR, 3.0–4.7) in those with 214L. In the multivariable survival analysis, the detection of polymorphism 214L versus 214F before initiation of the TA-containing cART regimen was significantly associated with a lower risk of virological failure (adjusted RH, 0.63 [95% CI, 0.42–0.96];  $P = .03$ ). Interestingly, in this survival analysis we found no evidence for a difference in risk of virological failure associated with the detection of polymorphism 214L according to concomitant detection of TAMs and TAM profiles (*P* values for the test of interactions were as follows: for 41L,  $P = .07$ ; for 215Y,  $P = .33$ , and for mixed profiles,  $P = .22$ ). In contrast, in the linear regression analysis, a larger difference in week 24 VL reductions between patients with 214L and those with 214F appeared to be in patients who concomitantly harbored 41L, 215Y, or mixed profiles before the initiation of the TA-containing cART (table 3). The *P* values for the test of interactions were as follows: for M41L,  $P = .03$ ; for E44A/D,  $P = .25$ ; for D67N,  $P = .51$ ; for K70R,  $P = .59$ ; for V118I,  $P = .90$ ; for L210W,  $P = .19$ ; for T215Y,  $P = .001$ ; for K219E/Q,  $P = .97$ ; for type 1 TAMs,  $P = .56$ ; for type 2 TAMs,  $P = .91$ ; and for mixed profiles,  $P = .02$ .

When the overall effect of TAMs on virological response was analyzed, compared with that in patients in whom no TAMs were detected, the mean reductions in VL were +0.65 (95%

CI, +0.22 to +1.07)  $\log_{10}$  copies/mL for those with type 1 TAMs ( $P = .003$ ), +0.54 (95% CI, +0.02 to +1.05)  $\log_{10}$  copies/mL for those with type 2 TAMs ( $P = .04$ ), and +0.71 (95% CI, +0.22 to +1.20)  $\log_{10}$  copies/mL for those with mixed profiles ( $P = .005$ ).

Again, the on-treatment analysis provided similar results (data not shown). Similar results both in ART-naïve and ART-experienced patients were found when the analysis was restricted to those who initiated a PI-based cART regimen (RH, 0.36 [95% CI, 0.19–0.68];  $P = .002$ ).

**HIV evolution according to the detection of F214L polymorphism.** Finally, to test whether the detection of F214L before therapy was associated with the subsequent observation of a specific TAM pathway, we performed an analysis in the subsets of patients who had an additional genotype available after starting therapy and in whom specific TAMs could not be detected by genotypic testing before starting the TA-containing cART. Overall, the median time from the date of starting therapy to this second test was 18 (range, 2–88) months. In these subsets (for which the sample size ranged from  $n = 135$  in the 215Y development analysis to  $n = 221$  in the 219E/Q development analysis), the proportion of patients who harbored the 214L variant was 17%. The proportions of people with 214L vs. those with 214F who appeared to have accumulated specific TAMs were as follows: for 41L, 4% versus 12%



**Figure 2.** Kaplan-Meier curves for probability of virological failure (2 consecutive viral load measurements >400 copies/mL after at least 6 months of thymidine analogue [TA]-containing combination antiretroviral therapy [cART]), according to the detection of variant L or F at position 214 of the reverse transcriptase region in ART-naïve patients starting TA-containing cART.

( $P = .47$ ); for 210W, 0% versus 7% ( $P = .36$ ); for 215Y, 4% versus 7% ( $P = 1.00$ ); for 67N, 13% versus 10% ( $P = .71$ ); for 70R, 14% versus 5% ( $P = .13$ ), and for 219E/Q, 9% versus 8% ( $P = 1.00$ ). These findings are consistent with a pathway agonistic to type 2 TAM profiles and antagonistic to type 1 TAM profiles in people harboring 214L at the pretherapy test. Finally, of 15 patients with no TAMs and 214L at the pretherapy test, 12 (80%) developed  $\geq 1$  TAM after treatment initiation; of 84 patients with no TAMs and 214F, 72 (86%) developed  $\geq 1$  TAM ( $P = .57$ ).

## DISCUSSION

This is, to our knowledge, the first large study providing evidence that the detection of polymorphism F214L may confer virological benefit to patients starting a zidovudine- or stavudine-containing cART. Larger week 24 VL reductions were observed in both ART-naïve and ART-experienced patients carrying F214L, and this persisted during more extended follow-up, as evidenced by a lower hazard of virological failure. These analyses were conducted according to an intention-to-treatment principle by ignoring changes in therapy; therefore, results may not be due to continued exposure to the TAs. However, results were similar when analyses were repeated using an on-treatment approach that censored events at the date of interruption of TAs.

Interestingly, the relevance of F214L for virological response to combination therapy has already been suggested in 2 pre-

vious studies. In 55 patients enrolled in the ACTG241 trial, mutations in baseline sequences at positions 214, 196, and 200 were associated with a stronger response to didanosine-zidovudine-containing therapy at week 48 [28]. Similarly, in 111 patients enrolled in the Jaguar trial, F214L was associated with a better virological response to didanosine at week 4 [26]. However, in our analysis, we did not find evidence of a different association between 214L/F and virological outcome according to whether didanosine was or was not used in the regimen (data not shown), suggesting that a favorable response to TA-containing regimens may be expected irrespective of which other NRTI is used.

The benefit of virological response associated with the detection of F214L versus 214F in the HIV-1 RT may be partially due to the strong negative association between F214L and type 1 TAMs [20, 26, 27]. Although the association between 214L and virological response to TA-containing cART was independent of predicted TA activity at baseline, it is conceivable that the particular dominant variant at position 214 before the initiation of therapy may represent one of the determinants for TAM pathway choice; thus, 214L, by inhibiting the development of type 1 TAMs or a large number of TAMs (as in mixed profiles), may provide a favorable effect on virological response. Generally, the prevalence of type 1 TAMs is higher than that of type 2 TAMs in NRTI-treated patients [9, 12–14, 29]. In agreement with these data, in our cohort of ART-experienced patients, the prevalence of type 1 TAM profiles was much higher

**Table 2. Adjusted relative hazards (RHs) of virological failure from fitting a proportional hazards Cox regression model for antiretroviral therapy (ART)–naïve and ART-experienced patients.**

Factor	ART naïve		ART experienced	
	RH (95% CI)	<i>P</i>	RH (95% CI)	<i>P</i>
Age, per 10 years older	0.77 (0.58–1.01)	.06	0.91 (0.78–1.08)	.28
RT214				
F	1.00		1.00	
L	0.22 (0.07–0.72)	.01	0.63 (0.42–0.96)	.03
Viral load, per log <sub>10</sub> copies/mL	1.13 (0.77–1.66)	.52	1.26 (1.06–1.50)	.008
Susceptibility to thymidine analogue <sup>a</sup>				
Susceptible	1.00		1.00	
Intermediate	3.45 (0.32–37.6)	.31	1.08 (0.72–1.64)	.70
Resistance	2.43 (0.31–19.3)	.40	1.08 (0.78–1.50)	.64
No. of active drugs <sup>a</sup> in regimen besides zidovudine/stavudine, per additional drug	0.57 (0.28–1.19)	.04	0.72 (0.58–0.91)	.005
Time from test to cART initiation, per month longer	1.14 (1.00–1.31)	.05	1.05 (0.97–1.13)	.25

**NOTE.** RHs are adjusted for sex, mode of HIV transmission, exact no. of weeks between the date of genotypic test and the date of starting combination ART (cART), whether a patient started zidovudine- or stavudine-containing cART, previous use of thymidine analogues as suboptimal therapy, use of dideoxyinosine, and third drug used (with efavirenz as the comparator) at the time of starting zidovudine or stavudine therapy. CI, confidence interval; RT, reverse transcriptase.

<sup>a</sup> Per the Rega Institute interpretation system (version 6.4.1).

than that of type 2 TAM profiles irrespective of the concomitant detection of F214L (37% vs. 15% with the simple definition; 43% vs. 18% with the strict definition;  $P < .0001$ ). Our analysis also confirms, as previously observed in other studies [20, 26], that F214L in ART-experienced patients is strongly negatively associated with M41L, L210W, T215Y, type 1 TAM profiles, and mixed TAM profiles and is positively associated with type 2 TAM profiles. A stabilizing mechanism explained by the structural vicinity of positions 214 and type 1 TAMs 215 and 210 may drive this clustering phenomenon [20] (F.C.-S., A. Artese [University of Catanzaro Magna Grascia, Roccelletta di Borgia, Italy], S. Alcaro [University of Catanzaro Magna Grascia, Roccelletta di Borgia, Italy], and C.F.P., unpublished data). Indeed, a background of 214F (instead of 214L), because of the interaction of the aromatic side chains, may favor and stabilize the enzyme of virus populations carrying mutation T215Y, which is normally the first occurring type 1 TAM [30], and the following L210W (F, W, and Y contain aromatic side chains); in contrast, polymorphism 214L does not have aromatic side chains and, therefore, may not increase the stability of the 3-dimensional structure of the enzyme in the presence of type 1 TAMs.

A low stability of RT enzymes harboring 214L and type 1 TAMs and mixed TAM profiles may induce a low level of viral replication and also explain the markedly better virological response to TA-containing cART observed in patients with 214L than in those with 214F when M41L, T215Y, or, in general, a type 1 TAM or mixed TAM profile was concomitantly detected. Consistent with this hypothesis, it has been proposed that virus populations carrying a type 1 TAM profile are less susceptible

to zidovudine and stavudine in the presence of 214F instead of 214L [27] and that 214F may improve the efficacy of the ATP-mediated removal of the zidovudine and stavudine monophosphate from the terminated cDNA chain [31]. Finally, in 2 separate analyses of genotypic-phenotypic correlations, F214L was associated with an increased susceptibility to didanosine [32] and tenofovir [33].

Therefore, all of these data suggest that F214L, either alone or in combination with specific mutations, may alter the RT structure, impacting viral fitness and/or drug susceptibility and thus affecting the response to TAs as well as to other antiretrovirals. More phenotypic and clinical data are necessary to confirm these observations, and the exact molecular mechanism responsible for the action of polymorphism F214L needs to be further investigated. For example, it is still unclear whether the course of HIV-1 evolution (from wild type to type 1 TAMs and type 2 TAMs) under pressure of a TA can be influenced by the presence of such a polymorphism, either alone or with other mutations and/or factors. In our analysis, even if the results were not statistically significant (possibly due to the lack of power; the maximum sample size was 221), a trend toward a greater accumulation of type 2 TAMs was observed in patients in whom the 214L variant instead of the 214F variant was detected at baseline. These data are in agreement with those of another recent study [34].

Overall, even if this is a large study focusing on F214L and virological response, a limitation is the fact that a longitudinal analysis using >1 genotype per patient could be performed only in a small subset of patients, not all of whom were drug naïve. We cannot exclude the possibility that past suboptimal mono-



**Table 3. Average difference in week 24 viral load reduction between patients with variant 214L or 214F, according to specific mutations and to mutation profiles concomitantly detected.**

	214L vs. 214F		
	Crude difference, mean (95% CI)	Adjusted difference <sup>a</sup>	
Mutation		Mean (95% CI)	<i>P</i> for interaction
RT mutation			
41			
M	0.20 (−0.42 to 0.81)	−0.16 (−0.77 to 0.46)	
L	0.94 (0.06 to 1.82)	0.83 (0.09 to 1.57)	.03
215			
T	0.05 (−0.54 to 0.64)	−0.08 (−0.66 to 0.49)	
Y	1.13 (0.16 to 2.09)	1.02 (0.20 to 1.85)	.001
TAM profile			
No TAMs	0.25 (−0.56 to 1.06)	0.04 (−0.78 to 0.87)	
Type 1 TAM <sup>b</sup>	0.26 (−0.92 to 1.44)	0.52 (−0.43 to 1.46)	.56
Type 2 TAM <sup>b</sup>	−0.35 (−1.25 to 0.56)	−1.38 (−2.22 to −0.52)	.91
Mixed TAM	1.51 (0.40 to 2.62)	1.35 (0.45 to 2.26)	.02

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. CI, confidence interval; RT, reverse transcriptase; TAM, thymidine analogue mutation.

<sup>a</sup> Adjusted for sex, age, mode of HIV transmission, pre-combination antiretroviral therapy (cART) viral load, exact no. of weeks between the date of genotypic test and the date of starting cART, whether a patient started a zidovudine- or stavudine-containing cART, whether zidovudine or stavudine were active (according to the Rega Institute interpretation system [version 6.4.1]), the no. of drugs besides zidovudine and stavudine to which virus was susceptible (according to Rega), previous use of thymidine analogues as suboptimal therapy, use of dideoxyinosine, the third drug used (with efavirenz as the comparator) at the time of starting zidovudine or stavudine therapy, and the exact no. of weeks between the date of starting cART and the week 24 viral load measurement.

<sup>b</sup> Simple definition.

therapy or dual therapy with TAs may have already influenced the evolution of minor variants without apparently selecting TAMs before the initiation of cART. Therefore, further investigations using larger databases are warranted to confirm whether the detection of F214L in drug-naïve patients favors the selection of type 2 TAMs versus type 1 TAMs. Indeed, this issue is of high relevance for researchers and clinicians, because these 2 distinct TAM clusters have diverse clinical significance: type 1 TAMs are associated with higher phenotypic resistance to zidovudine and higher cross-resistance to other NRTIs (such as didanosine and tenofovir) than type 2 TAMs [15, 16, 29, 35–37]. A type 1 TAM profile is associated with an impaired virological response to tenofovir-containing therapy and with a 1.8-fold increase in the risk of disease progression and/or death, whereas a type 2 TAM profile does not have a detrimental effect [16, 17, 38, 39].

The prevalence of 214F/L was not different according to HIV subtype in our study. This is inconsistent with what has been found in other study populations. For instance, the prevalence of 214L in drug-naïve patients varies from 2.5% (subtype AE) to 17.8% (subtype D) [5]. Therefore, further investigation of the virological response to TA-based cART according to the detection of 214L in patients carrying non-B subtypes is warranted.

In conclusion, the present study provides evidence that patients with the natural polymorphism F214L have a better virological response to TA-containing cART than those with 214F. Sequencing the RT region is currently recommended in treatment-naïve patients about to start therapy if the suspicion of transmitted resistance is high or if its prevalence in the population exceeds 10% [40]. Therapy choice might benefit from taking polymorphisms at codon 214 into account as potential contributors to the future course of resistance evolution and response to first-line treatment with zidovudine. The considerable prevalence of this polymorphism (16%–18%) and its relevance at the time of treatment selection strongly argue in favor of extended the genotyping of all patients starting ART.

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